

Bennett, John

From: Fout, Shay
Sent: Thursday, February 19, 2015 9:30 AM
To: 'Li, Charles'
Subject: RE: Water Cluster Project-EMSL Kit arrival time

Hi Charlie,

Thanks for the reply. I will try to do some follow-up on the remaining reagents, but it probably won't be until late next month or early April. Do you have any objection to co-writing a short note on comparing the kit with the standard assay if all the remaining assays give good results. If so, would we need to and how could we protect EMSL's ability to produce and sell kits if it ever became economically feasible?

Shay

G. Shay Fout, Ph.D.
Senior Research Microbiologist
U.S. Environmental Protection Agency (MS 593)
Office of Research and Development
National Exposure Research Laboratory
Microbiological & Chemical Exposure
Assessment Research Division
Biohazard Assessment Research Branch
26 W. Martin Luther King Jr. Drive
Cincinnati, OH 45268
Phone: 513-569-7387
Fax: 513-569-7464
Email: fout.shay@epa.gov

From: Li, Charles [<mailto:cli@EMSL.com>]
Sent: Thursday, February 19, 2015 8:54 AM
To: Fout, Shay
Subject: RE: Water Cluster Project-EMSL Kit arrival time

Hi, Shay,

I did not add anything, I followed your SOW closely. I think that you are right, the primers and probes, armored Hepatitis G were all freshly made and I dissolved and used within a week. In my experiences, fresh primers and probes do work better than -80C freezer stored for sometimes.

Would you try to use the reagents I sent back to you? They are in the box.

Thanks

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]
Sent: Thursday, February 19, 2015 8:36 AM
To: Li, Charles
Subject: RE: Water Cluster Project-EMSL Kit arrival time

Hi Charlie,

We have not finished the analysis of the kit versus standard assays yet, but I wanted to give you an update. We decided that with everyone's tight schedules and with wanting to use the same thermal cycler for both the standard and kit assays to avoid any difference between thermal cyclers, the assays could not be run on the week that the kits arrived. Instead, we did the RT assays last week and are doing the hepatitis G assays this week. Next week we will do the enterovirus and norovirus assays. We are running the last of the hepatitis G assays today, but have results from most of the samples.

Surprisingly, the kits are outperforming the standard assay by an average of 1.3 (first set of plates) to 2.3 (second set) Cq values on the hepatitis G plates. Did you add any stabilizers to the kit ingredients that could explain the superior performance of the kits? The only other difference I can think of is that we made our primer and probe dilutions after receiving them last year and they have been stored at -20°C along with the ROX, rather than at -80 like all other reagents. We did a pretest on some of the samples at that time to make certain there was enough virus in the samples to detect. Can you think of any other reason why the kit assays would be better than the standard assays?

Best regards,
Shay

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From: Li, Charles [<mailto:cli@EMSL.com>]
Sent: Wednesday, February 04, 2015 11:41 AM
To: Fout, Shay
Cc: Hyde, Joe
Subject: RE: Water Cluster Project-EMSL Kit arrival time

Hi, Shay,

Is everything Ok for the package? Joe told me that the delivery arrived your site.

Thanks

Charlie



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From: Li, Charles

Sent: Tuesday, February 03, 2015 4:05 PM

To: 'Fout, Shay'

Subject: RE: Water Cluster Project-EMSL Kit arrival time

Hi, Shay,

The FedEx tracking number is 772812152130, priority overnight, will arrive in the morning.

Thanks

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]

Sent: Tuesday, February 03, 2015 6:54 AM

To: Li, Charles

Subject: RE: Water Cluster Project

Charlie,

Please ship to G. Shay Fout,

26 W. Martin Luther King Jr. Drive, Cincinnati, OH 45268. Thanks,

Shay

G. Shay Fout, Ph.D.

Senior Research Microbiologist

U.S. Environmental Protection Agency (MS 593)

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From: Li, Charles [<mailto:cli@EMSL.com>]
Sent: Monday, February 02, 2015 5:02 PM
To: Fout, Shay
Cc: Hyde, Joe
Subject: RE: Water Cluster Project
Importance: High

Hi, Shay,

Can you provide the shipping address? We will send the package to you tomorrow via FedEx and you will receive it on Wednesday.

Thanks

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]
Sent: Thursday, January 29, 2015 8:09 AM
To: Li, Charles
Subject: RE: Water Cluster Project

Hi Charlie,

Have you decided what day next week we will be receiving the kits? I have a meeting with my group this afternoon to work out the schedule for processing them, and will need to take into account the day we receive them. It would be best if we could get them on Wednesday, as it will take at least two days to run all the plates, and because a lot of the staff are on a compressed work schedule and take Fridays off. However, if you can't get them to us until Thursday, I will try to make it work, but we may have to wait until Monday to run them.

Thanks,
Shay

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From: Li, Charles [<mailto:cli@EMSL.com>]
Sent: Thursday, January 22, 2015 9:21 AM
To: Fout, Shay
Subject: RE: Water Cluster Project

Hi, Shay,

When is it the best time to call you? I think we need go over it on the phone.

Thanks

Charlie

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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]
Sent: Thursday, January 22, 2015 8:30 AM
To: Li, Charles
Subject: RE: Water Cluster Project

Hi Charlie,
The SOP look fine, except for the following.

Table 8:

- A. The Armored RNA for Method 1615 contains the RNA for all the reactions, i.e., the enterovirus and norovirus GI and GII sequences, so there is no need to set up separate RT assays for each.
- B. What do you mean by the - Extra HGV RNA column? If you mean that you want to run a separate RT set without HGV Armored RNA, then you would need to add 15.5 µL of mix and 1 µL of extra water to compensate. If you don't mean this, then this column is not needed, because all assay have HGV RNA, i.e., I think there should just be two columns in Table 8, one without and one with the Method 1615 Armored RNA. Thus the table should be as follows:

Steps	RT Reaction	Negative	Positive
1	RT Master Mix 1	16.5 µL	16.5 µL
2	dH ₂ O	6.7 µL	
3	Armored RNA EPA-1615		6.7 µL
4	Vortex – 10 sec	Yes	Yes
5	Spin – 10 sec	Yes	Yes
6	PCR Program-Annealing run*	Yes	Yes
7	Spin – 10 sec	Yes	Yes
8	RT Master Mix 2	16.8 µL	16.8 µL
9	Vortex – 10 sec	Yes	Yes
10	Spin – 10 sec	Yes	Yes
11	PCR Program RT Run**	Yes	Yes

Table 9: because all assays contain the HGV RNA, you should have one more column and the table should be as follows:

Assay	HGV		EV		GIB		GII	
	1	2	3	4	5	6	7	8
A	-RNA*	+RNA	-RNA	+RNA	-RNA	+RNA	-RNA	+RNA
B	-RNA	+RNA	-RNA	+RNA	-RNA	+RNA	-RNA	+RNA
C	-RNA	+RNA	-RNA	+RNA	-RNA	+RNA	-RNA	+RNA
D	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC
E	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC
F	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC

* -RNA is from the negative RT assay in Table 8 and +RNA is from the positive RT assay in Table 8.

Note that both the -RNA and +RNA wells should be positive in the HGV assay.

Would it be possible to set up the plates exactly as in the protocol I sent, i.e., not fill the plates completely, but add the appropriate mixes to just the wells indicated in the SOP; and then send us any remaining mixes left over after the QC? That would allow us to re-run a few samples, should it be necessary. If you fill up the wells completely, then any well not used would not be available for a re-run.

Please let me know if you have any questions or if I'm missing something.

Thanks,
Shay

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From: Li, Charles [<mailto:cli@EMSL.com>]
Sent: Thursday, January 08, 2015 4:12 PM
To: Fout, Shay
Subject: RE: Water Cluster Project

Hi, Shay,

Attached is my working SOP for the project. Please check if you can approve it before I use it.

Many thanks

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]
Sent: Thursday, January 08, 2015 10:49 AM
To: Li, Charles
Subject: RE: Water Cluster Project

Yes, go ahead and run the QC on EV, GIB and GII using the armored RNA standard. Please run a QC on the HGV as well, but please check to make certain I am sending you sufficient reagents for the QC tests. I was originally thinking that we would run the QC here using the standard curve as the positive QC and no template controls as the negative QC and only included enough reagents to make the plates; however, it certainly would be best for you to make certain all mixes are good before making the plates.

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Email: fout.shay@epa.gov

From: Li, Charles [<mailto:cli@EMSL.com>]
Sent: Thursday, January 08, 2015 10:34 AM
To: Fout, Shay
Subject: RE: Water Cluster Project

Hi, Shay,

Thanks. Yes, all primers and probes are in the supplies for making the kits. Shall I run QC tests for EV, GIB and GII using Armored RNA? I understand HGV has been used as QC in the RT-qPCR.

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]

Sent: Thursday, January 08, 2015 10:31 AM

To: Li, Charles

Subject: RE: Water Cluster Project

Hi Charlie,

I'm sending all the primers and probes and can send a vial of the armored RNA in addition. The armored RNA has a titer of 2.5×10^8 genomic copies per mL in a volume of about 0.25 mL.

Shay

G. Shay Fout, Ph.D.

Senior Research Microbiologist

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Email: fout.shay@epa.gov

From: Li, Charles [<mailto:cli@EMSL.com>]

Sent: Thursday, January 08, 2015 9:03 AM

To: Fout, Shay

Subject: RE: Water Cluster Project

Hi, Shay,

Will you provide QC reagents for the RT-qPCR kits? I need to run QC on Master Mixes, so I need qPCR primers and probes, and viral RNA or armored RNA as controls, the master mixes must show detections for viral RNAs, no contamination before I put the mixes into qPCR plates.

Thanks

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]

Sent: Wednesday, January 07, 2015 2:01 PM

To: Li, Charles

Subject: RE: Water Cluster Project

Hi Charlie,

I still don't have the dry ice, so it will have to wait until I return on the 20th. I'm at work this week through Friday. Thanks, Shay

G. Shay Fout, Ph.D.

Senior Research Microbiologist

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Phone: 513-569-7387

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Email: fout.shay@epa.gov

From: Li, Charles [<mailto:cli@EMSL.com>]

Sent: Wednesday, January 07, 2015 1:35 PM

To: Fout, Shay

Subject: RE: Water Cluster Project

Hi, Shay,

I have StepOnePlus and the 96 well plates. You do not need to send. I can wait when you return. I am going to send the protocol-my version to you soon, so you can review it.

Thanks

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]

Sent: Wednesday, January 07, 2015 1:25 PM

To: Li, Charles

Subject: RE: Water Cluster Project

Charlie,

We will use generic 96 well plates for the RT step and the One Step Plus System for the PCR step. This instrument uses the MicroAmp Fast Optical 96-Well Reaction Plates. I can send you some of each tomorrow. Attached is the latest version of the SOW which reflects some change from the last version I sent. It also shows what I will be sending you in terms of reagents. I may not get the reagents sent out today, as my dry ice that I was saving has evaporated. One of my colleagues got some packages in and there may be enough to use, but I have to leave at 3 today for a doctor's appointment, so there may not be enough time.

Shay

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Email: fout.shay@epa.gov

From: Li, Charles [<mailto:cli@EMSL.com>]

Sent: Wednesday, January 07, 2015 11:56 AM

To: Fout, Shay

Subject: RE: Water Cluster Project

Hi, Shay,

Which type of 96-well plates do you use in your lab? I know they are instrument specific. I will send you a working protocol for approval soon.

Thanks

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]

Sent: Tuesday, January 06, 2015 1:59 PM

To: Li, Charles

Subject: RE: Water Cluster Project

Hi Charlie,

I had hoped to send you the reagents yesterday, but have to get a paper resubmitted this week. The first author has been out of the country and did not have the ability to do the edits; the journal said we have to get it back or we would have to resubmit. I had to take over the edits and am almost finished, but won't have time to get everything together today. Will you be in on Friday? If so, I will try to send the package tomorrow. If not, I'm out of the office all next week, so it would have to wait until 20th before I could send it.

Happy New Year.

Shay

G. Shay Fout, Ph.D.

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Email: fout.shay@epa.gov

From: Li, Charles [<mailto:cli@EMSL.com>]

Sent: Tuesday, December 09, 2014 9:09 AM

To: Fout, Shay

Subject: RE: Water Cluster Project

Hi, Shay,

Winter season is good time for me to work with you on research projects. I will work on the kits when the reagents arrive.

Thanks & Happy Holiday to You!

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]

Sent: Tuesday, December 09, 2014 7:53 AM

To: Li, Charles

Subject: RE: Water Cluster Project

Hi Charlie,

I apologize for the delay in follow-up to our last email exchange. The last couple of months have been hectic, especially early in the week. I meant to send you the reagents before now, but with the holidays, I think it would be best to wait until after the 1st, thus I send them by overnight delivery on 1/5/15. We have collected most of the samples needed for the kit testing and will finish the collection next week, and then do extractions on 1/7. We can then do the kit testing at your convenience in late January or during February. I will contact you when I send the reagents and get your time frame for the kit preparation.

Have a Happy Holiday season,
Shay

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From: Li, Charles [<mailto:cli@EMSL.com>]

Sent: Thursday, October 23, 2014 8:51 AM

To: Fout, Shay

Subject: RE: Water Cluster Project

Hi, Shay,

That is fine. Thanks

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]

Sent: Thursday, October 23, 2014 8:45 AM

To: Li, Charles

Subject: RE: Water Cluster Project

Hi Charlie,

Everyone in my Branch has been too tied up with other projects to get the samples completed over the last two months, but it looks like we will be able to complete them during November. December will probably be too busy to do the analyses, so I'm thinking about targeting the method and kit analyses for January. Will that be workable for your group?

It also took longer than I expected to get all the reagents, but I have received them and will be sending them to you either next week or the week after.

Thanks and best regards,
Shay

G. Shay Fout, Ph.D.
Senior Research Microbiologist
U.S. Environmental Protection Agency (MS 593)
Microbiological & Chemical Exposure
Assessment Research Division
Biohazard Assessment Research Branch
26 W. Martin Luther King Jr. Drive
Cincinnati, OH 45268
Phone: 513-569-7387
Fax: 513-569-7464
Email: fout.shay@epa.gov

From: Li, Charles [<mailto:cli@EMSL.com>]

Sent: Wednesday, August 27, 2014 9:32 AM

To: Fout, Shay

Subject: RE: Water Cluster Project

Hi, Shay,

There is no problem from my end. I will make your project as top priority and I have analysts working with me on routine samples.

Thanks

Charlie



Charles Li, Ph.D. | *Director of PCR and DNA Analysis Laboratory*

EMSL Analytical, Inc. | 200 Route 130 North | Cinnaminson, NJ 08077

Phone: 856-303-2554 | Fax: 856-786-0262 | Toll Free: 800-220-3675

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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]

Sent: Wednesday, August 27, 2014 9:17 AM

To: Li, Charles

Subject: RE: Water Cluster Project

Hi, Dr. Li,

I want to give you an update of our status. I have ordered all the supplies needed and will send you portions to you next week, assuming everything arrives.

The schedule for collecting the samples is slipping from what I had originally anticipated. We have collected and processed six reagent grade water samples (seeded with primary effluent), but have decided that we need to run a pretest on a couple of them using the new reagents before collecting the remaining sample sets. We want to make certain that there is enough virus in the amount of seed added to give decent Ct values. In addition, the staff are overwhelmed with other project deadlines, so it will probably be the end of October or early November before we will be ready for the complete assays. This will delay your preparation of the test kits until about that time. Please let me know if there will be any problems with the delay.

Thanks and best regards,
Shay

G. Shay Fout, Ph.D.
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Fax: 513-569-7464
Email: fout.shay@epa.gov

From: Li, Charles [<mailto:cli@EMSL.com>]

Sent: Tuesday, July 15, 2014 4:43 PM

To: Fout, Shay

Subject: RE: Water Cluster Project

Hi, Dr. Fout,

I will work on them directly without delays, I think we are better to use in weeks, I will try to ship to you in the same week or next week.

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]

Sent: Tuesday, July 15, 2014 1:33 PM

To: Li, Charles

Subject: RE: Water Cluster Project

Hi Dr. Li,

I failed to mention that it will take time to collect and process all the samples mentioned in the statement of work. I have a meeting with my EPA team tomorrow, so should have a better feel for how long, but it will probably take a couple of months to get all of them ready. What is your experience with shelf life of the plates you prepare? Can we go ahead and have you prepare the plates as soon as we get funding and expect them to be fine if there is a several month delay before they are used?

Best regards,

Shay

G. Shay Fout, Ph.D.

Senior Research Microbiologist

U.S. Environmental Protection Agency (MS 593)

Microbiological & Chemical Exposure

Assessment Research Division

Biohazard Assessment Research Branch

26 W. Martin Luther King Jr. Drive

Cincinnati, OH 45268

Phone: 513-569-7387

Fax: 513-569-7464

Email: fout.shay@epa.gov

From: Li, Charles [<mailto:cli@EMSL.com>]

Sent: Tuesday, July 15, 2014 12:29 PM

To: Fout, Shay; Dombro, Danielle

Cc: Dobranic, Jason; Grimm, Ann

Subject: RE: Water Cluster Project

Hi, Dr. Fout,

We consider EPA research project as our top priority work, we have adequate resources to cover the work listed. We will meet your completion time requirement.

Thanks

Charlie



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From: Fout, Shay [<mailto:Fout.Shay@epa.gov>]

Sent: Tuesday, July 15, 2014 11:28 AM

To: Li, Charles; Dombro, Danielle

Cc: Dobranic, Jason; Grimm, Ann

Subject: RE: Water Cluster Project

Hi Dr. Li,

Please find attached the signed UBMTA with the complete Statement of Work. The funds that will be used to purchase the necessary reagents have to be transferred from one EPA account to another. I'm not certain how long that will take, but will let you know when I can start ordering supplies. Is there a particular time over the next 1-3 months when you would like to start your part of the cooperative effort?

I look forward to working with you.

Shay

G. Shay Fout, Ph.D.

Senior Research Microbiologist

U.S. Environmental Protection Agency (MS 593)

Microbiological & Chemical Exposure

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26 W. Martin Luther King Jr. Drive

Cincinnati, OH 45268

Phone: 513-569-7387

Fax: 513-569-7464

Email: fout.shay@epa.gov

From: Li, Charles [<mailto:cli@EMSL.com>]

Sent: Tuesday, July 15, 2014 10:39 AM

To: Fout, Shay

Subject: RE: Water Cluster Project

Hi, Dr. Fout,

I read the SOP and I do not see any problem to follow it and make the plates and reagents mix for you.

Let me know what you need from me.

Thanks

Charlie



Charles Li, Ph.D. | *Director of PCR and DNA Analysis Laboratory*

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